II. REMARKS

Upon entry of the amendment, claims 1, 3, 4, and 15 to 19, and 20 to 23 will be pending.

A. Regarding the Amendments

Claims 20 and 24 and, pursuant to the Restriction Requirement, claims 5 to 14, are cancelled herein without disclaimer, and without prejudice to Applicants' pursuing prosecution of subject matter encompassed within one or more of the claims in an application claiming the benefit of priority of the subject application.

Claim 1 has been amended to clarify that the "hydrophobic region" of the polypeptides of the invention is a "hydrophobic core region comprises a heptad leucine zipper domain, which forms an α-helix." The amendment is supported, for example, at page 4, lines 16-25, and page 9, line 23, to page 10, line 4 (see, also, page 2, line 24, to page 3, line 2; and Figure 1C). As such, the amendment does not add new matter.

Claim 1 also has been amended to clarify that the non-natural amino acid "replaces a hydrophobic amino acid in the heptad leucine zipper domain". The amendment is supported, for example, at page 8, lines 12-14, and page 11, lines 6-9, and therefore, does not add new matter.

Claims 16 and 17 have been amended such that the language of the claims conforms to that of amended claim 1, from which claims 16 and 17 depend. Claim 22 has been amended to correct a typographical error. As such, these amendments address a formality or correct a readily apparent typographical error and, therefore, do not add new matter.

It is submitted that the amendments do not require a new search or new consideration because the amendment to claim 1 incorporates the subject matter of previously pending

claims 22 and 24, which have been under consideration. The amendments were not made earlier in the prosecution because it was believed that the previous amendments and remarks addressed the outstanding issues. It is submitted that the amendments place the claims in condition for allowance, or in better condition for appeal. For these reasons, and because the amendments do not add new matter, it is respectfully requested that the amendments be entered.

B. Rejection under 35 U.S.C. § 112

The objection to the specification and corresponding rejection of claims 1, 3 and 4 under 35 U.S.C. § 112, first paragraph, as allegedly lacking an adequate written description are respectfully traversed.

It is maintained in the Office Action the specification does not provide a sufficiently representative number of exemplary polypeptides of the invention to support the breadth of the claimed subject matter. It is stated, for example, that the specification discloses two polypeptides, GCN4 and A1, incorporating non-natural amino acids, but does not provide examples of interleukins, tumor necrosis factor, or other polypeptides. In addition, it is stated that the claims refer to "hydrophobic regions" of polypeptides, but that it is unclear what constitutes a hydrophobic region (e.g., whether two hydrophobic amino acids next to each other constitute an hydrophobic region).

Claim 1 has been amended to more clearly indicate that non-natural amino acids are inserted into a "hydrophobic core region comprising a heptad leucine zipper domain, which forms an α-helix." Applicants submit that leucine zipper domains are well known in the art, and that the GCN4 leucine zipper is recognized in the art as a model system for studying the leucine zipper motif (see, e.g., Hendsch and Tidor, *Prot. Sci.* 8:1381-1392, 1999, a copy of which is attached as Exhibit A - see first two sentences of Abstract, and page 382, left column, third full paragraph; see, also, Havranek and Harbury, *Nat. Struct. Biol.* 10:45-52, 2003, a copy of which is attached as Exhibit B - see page 46, left column, last full paragraph). As

such, it is submitted that one skilled in the art, viewing the specification, which discloses that substitution of a hydrophobic amino acid (leucine) with a non-natural amino acid in the heptad leucine zipper increases the thermal stability of the modified polypeptide (see, e.g., page 21, lines 4-8, and Figure 2B), would have known that the disclosed results would be predictive of similarly substituting a non-natural amino acid for a hydrophobic amino acid in other leucine zipper-containing polypeptides, because the GCN4 leucine zipper is an art recognized model for leucine zipper-containing polypeptides.

In support of Applicants' position that the results obtained using the GCN4 peptide is generally predictive with respect to leucine zipper containing proteins, it is noted that the specification further discloses that insertion of non-natural amino acids for hydrophobic amino acids in the protein A1 heptad leucine zipper domains similarly increased the thermal stability of the modified A1 proteins (see, e.g., page 29, lines 21-29, and Figures 4A and 7). Thus, the results disclosed for the A1 polypeptide provides confirmatory evidence that, as disclosed for GCN4, substitution of a hydrophobic amino acid with a non-natural amino acid in a heptad leucine zipper domain of a polypeptide can increase the thermal stability of the polypeptide. In this respect, it is noted that, in addition to transcription factors such as GCN4, leucine zipper domains are present in a variety of other proteins, including, for example, in enzymes (see, e.g., Zulfiqar et al., *J. Biol. Chem.* 277:9226-9232, 2002, a copy of which is attached as Exhibit C - see Abstract; and Chiravuri et al., *J. Biol. Chem.* 275:26994-26999, 2000, a copy of which is attached as Exhibit D - see Abstract) and in membrane proteins (see, e.g., Gurezka et al., *J. Biol. Chem.* 274:9265-9270, 1999, a copy of which is attached as Exhibit E - see Abstract, and Table I at page 9268).

In view of the results disclosed in the subject application, as well as of knowledge in the art that the GCN4 leucine zipper domain is prototypical of the abcdefg heptad characteristic of this class of coiled coil proteins (see Exhibit B, page 46, left column, last full paragraph), it is submitted that the skilled artisan, viewing the subject application, would have

known that Applicants were in possession of the subject matter claimed as the invention. Accordingly, it is respectfully requested that the objection to the specification be withdrawn, and that the corresponding rejection of the claims under 35 U.S.C. § 112, first paragraph, be removed.

C. Prior Art Rejections

The rejection of claims 1, 3, 4, 15, 16 and 23 under 35 U.S.C. § 102(b), as allegedly being anticipated by Rennert et al. is respectfully traversed.

It is stated in the Office Action that Rennert et al. describe growing *E. coli* in the presence of 5',5',5'-trifluoroleucine, which would be incorporated into proteins, and that such proteins would inherently have increased thermal stability of the proteins as compared to a corresponding wild type polypeptide. Applicants submit, however, that Rennert et al. do not teach or suggest an "isolated" polypeptide as required by the claims, but only describe an extracted protein residue of *E. coli* obtained following TCA extraction and ethanol precipitation (see page 472, paragraph bridging columns). As such, it is submitted that the reference does not teach or suggest the claimed polypeptides and, therefore, respectfully requested that the rejection of the claims as anticipated by Rennert et al. be removed.

The rejection of claims 1, 3, 4, 15, 16 and 23 under 35 U.S.C. § 102(b) as allegedly being anticipated by Russell et al. is respectfully traversed.

It is stated in the Office Action that Russell et al. describe gramicidin A in which valine was chemically replaced by trifluorovaline or hexafluorovaline, and that such a substitution inherently would increase thermal stability of the modified gramicidin A. Applicants submit, however, that gramicidin A does not comprise a hydrophobic core region comprising a heptad leucine zipper domain, which forms an α-helix, as required by the claims. Leucine zipper domains are characterized, in part, in that they form a α-helical structure that is involved in protein-protein interactions, including, for example, homodimers

and heterodimers (see, e.g., Exhibit A, page 1382, left column, third full paragraph, and Figure 1; see, also, Exhibit C, page 9226, paragraph bridging columns, and page 9231, paragraph bridging columns; and Exhibit D, Abstract). In contrast, gramicidin A is a 15 amino acid peptide that forms a β-strand secondary structure (see, e.g., Kovacs et al., *Proc. Natl. Acad. Sci., USA* 96:7910-7915, 1999, a copy of which is attached as Exhibit F; see paragraph bridging pages 7912-7913, page 7914, paragraph bridging columns). Further, while gramicidin A can dimerize to form channels in membranes, the dimers are head-to-head β helical dimers (see, e.g., Tang et al., *Biophys. J.* 76:2346-2350, 1999, a copy of which is attached as Exhibit G; see page 2348, left column, first full paragraph). As such, gramicidin A does not comprise a hydrophobic core region comprising a heptad leucine zipper domain, which forms an α-helix, as required by the claims and, therefore, the teaching of Russell et al. does not anticipate the claimed polypeptides. Accordingly, it is respectfully requested that the rejection of the claims as anticipated by Russell et al. be removed.

The rejection of claims 1, 3, 4, 15, 16 and 23 under 35 U.S.C. § 102(b), as allegedly being anticipated by Arai et al. is respectfully traversed.

It is stated in the Office Action that Arai et al. describe replacing valine residues with L-hexafluorovaline (Hfv) residues in gramicidin S, and alleged that such a substitution inherently would increase thermal stability of the modified gramicidin S. Applicants point out, however, that gramicidin S is a cyclic decapeptide that contains two proline residues (see Arai et al., see page 1383, paragraph bridging columns, and Figure 1 at page 1384). As such, it is submitted that gramicidin S cannot form an α-helix because, as is well known, proline is an imino acid that disrupts secondary structure. As such, it is submitted that Arai et al. do not teach or suggest an isolated polypeptide as claimed and, therefore, respectfully requested that the rejection of the claims as anticipated by Arai et al. be removed.

The rejection of claims 1, 3, 4, and 16 under 35 U.S.C. § 102(b), as allegedly being anticipated by Mendel et al. is respectfully traversed.

It is stated in the Office Action that Mendel et al. describe replacing a leucine with S,S-2-amino-4-methylhexanoic acid at position 133 in T4 lysozyme, and alleged that such a substitution inherently would increase thermal stability of the modified lysozyme. It is noted in the Office Action that lysozyme residues 128 to 134 have the sequence AAVNLA, which comprises a hydrophobic region. Applicants point out that amino acid residues 126 to 134 (WDEAAVNLA) are known to comprise an α-helix in lysozyme (see Zhang et al., *Pro. Sci.* 1:761-776, 1992, a copy of which is attached hereto as Exhibit H; see Abstract, and Table 1 at page 763; see, also, Figure 12 at page 773). In this respect, it is noted that the α-helix in lysozyme is an octapeptide, not a heptad (heptapeptide) as is characteristic of leucine zipper domains and required by the claims. As such, it is submitted that Mendel et al. do not teach or suggest substituting a non-natural amino acid for a hydrophobic residue in a heptad leucine zipper domain and, therefore, do not anticipate the claimed polypeptides. Accordingly, it is respectfully requested that the rejection of the claims as anticipated by Mendel et al. be removed.

The rejection of claims 1, 3, 4, and 15 to 19 under 35 U.S.C. § 102(b), as allegedly being anticipated by Miyazawa et al. is respectfully traversed.

It is stated in the Office Action that Miyazawa et al. describe epidermal growth factor (EGF) in which Tyr-21 is replaced by norleucine, and that the Tyr residue is flanked by Met and Ile, thus constituting a hydrophobic region, and alleged that it would be inherent that the thermal stability of the modified EGF would be increased. Applicants point out, however, that the region of EGF comprising amino acids 19-23 (and 28-32) has an antiparallel β-sheet conformation (see, e.g., Lu et al., *J. Biol. Chem.* 276:34913-34917, 2001, a copy of which is attached as Exhibit I; see page 34915, right column, third paragraph). As such, it is submitted that Miyazawa et al. do not teach or suggest substituting a non-natural amino acid for a

hydrophobic amino acid in a hydrophobic core region comprising a heptad leucine zipper domain, which forms an α-helix, as required by the claims, and, therefore, do not anticipate the claimed polypeptides. Accordingly, it is respectfully requested that the rejection of the claims as anticipated by Miyazawa et al. be removed.

The rejection of claims 1, 3, 4, 15 to 19, and 22 under 35 U.S.C. § 102(b), as allegedly being anticipated by Koide et al. is respectfully traversed.

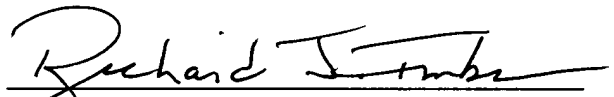
It is stated in the Office Action that Koide et al. describe EGF in which Tyr-22 and Tyr-29 are replaced by Phe, which is then modified, and that Tyr-21 (sic, Tyr-22), for example, is flanked by Met and Ile, thus constituting a hydrophobic region. It is alleged that it would be inherent that the thermal stability of the modified EGF would be increased. As discussed above, however, the region of EGF comprising amino acids 19-23 and 28-32 has an antiparallel β-sheet conformation (Exhibit I, page 34915, right column, third paragraph). As such, it is submitted that Koide et al. do not teach or suggest substituting a non-natural amino acid for a hydrophobic amino acid in a hydrophobic core region comprising a heptad leucine zipper domain, which forms an α-helix, as required by the claims, and, therefore, do not anticipate the claimed polypeptides. Accordingly, it is respectfully requested that the rejection of the claims as anticipated by Koide et al. be removed.

In view of the amendments and the above remarks, it is submitted that the claims are in condition for allowance, and a notice to that effect is respectfully requested. The Examiner is invited to call Applicants' undersigned representative if there are any questions relating to the subject application.

No additional fees other than those submitted herewith are believed necessary in connection with the filing of the communication. However, if any additional fees are deemed necessary, or if any overpayment has been made, the Commissioner is authorized to charge, or credit Deposit Account No. 50-1355.

Respectfully submitted,

Date: March 25, 2004



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